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OBSERVATIONS ON THE TOXICITY OF THREE ANTICHOLINESTERASE INSECTICIDES IN A VILLAGE-SCALE TRIAL AND COMPARISON OF METHODS USED FOR DETERMINING CHOLINESTERASE ACTIVITY*

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Two organophosphorus and one carbamate insecticide were investigated in a village-scale trial by WHO Insecticide Testing Unit in Lagos, Nigeria. Clinically no toxic effects were observed and only slight plasma cholinesterase depression was found in spraymen and in villagers exposed to one of insecticides tested.

During the trial comparison of four methods for cholinesterase determination was carried out. Their reliability in field conditions is discussed and each method is critically evaluated.

In view of the ever-increasing problem of insect resistance the introduction of new insecticides into field operations is one of the most essential prerequisites for the success of the malaria eradication programme. Since new insecticides as safe as DDT are unlikely to become available, extensive toxicological studies are required during the introduction of a new compound, which also include a trial under practical conditions on a village scale. The trial should provide sufficient toxicological information to decide whether or not it is advisable to proceed to the next stage i. e. an extended field trial.

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While the village trial can provide a basis for evaluations of the risk to the exposed villagers, it can hardly help to evaluate the risk to spraymen, as the spraying operation in a single village lasts a comparatively short time (1). Only an extended field trial – in the course of which the spraymen are exposed for several weeks – can provide reliable toxicological information about whether or not a new insecticide is recommendable for general use.

A village scale evaluation of three insecticides: two organophosphorus – *O,O*-dimethyl *O*-(4-bromo-2,5-dichlorophenyl)phosphorothioate (OMS–658) and *O,O*-dimethyl *O*-(4-nitro-*m*-tolyl) phosphorothioate (OMS–43) and one carbamate – 2-*iso*propoxyphenyl *N*-methyl carbamate (OMS–33) – was undertaken by WHO Insecticide Testing Unit in Lagos during 1964. Toxicological studies were performed to assess any adverse effects and significance of cholinesterase inhibition in spraymen and villagers exposed to the three insecticides.

This trial provided an opportunity to compare several methods of determining cholinesterase activity and to evaluate their reliability under field conditions.

MATERIALS AND METHODS

Exposure of spraymen and villagers to insecticides

Water dispersible powders of OMS–658, 43 and 33, were used containing 25, 40 and 50% of the active ingredient respectively. Three villages – Oko-Eko, Ewu-Elepe and Egbin – about 50 km north of Lagos were selected for spraying, each with one insecticide.

In the study of OMS–658 every effort was made to perform the experiment under conditions as similar as possible to those in experiments carried out by *Taylor* (2) on fenthion and by *Vandekar* (1) on OMS–43, so as to enable accurate comparison of the effects produced by three compounds. In the study of OMS–43, and especially of OMS–33 a prolonged period of spraying was planned, if possible, under weather conditions similar to those under which 3-*iso*propylphenyl *N*-methylcarbamate was sprayed in 1963 (1). However, the duration of spraying operations with OMS–658 and OMS–43 was limited by the amount of insecticides available.

Spraying with OMS–658 was performed by eight spraymen and two supervisors, and sprayings with OMS–43 and OMS–33 by another crew of three spraymen and one supervisor. A day or two prior to each spraying cycle the required amount of insecticide was weighed in small paper bags so that 5 percent of the active ingredient was obtained in the final spray. In accordance with the construction of the sprayer and spraying rhythm (19 m^2/min.) deposits of 2 g of active ingredient per square metre were expected.

Spraying was carried out on three separate occasions (Table 1): on the first (25th and 26th May) OMS–658 was sprayed for four and two-and-a-half hours, respectively; on the second (11th and 12th June) OMS–43 was sprayed for four to five hours a day. An additional one-hour-spraying of OMS–33 was performed by the remainder of the crew (eight spraymen who sprayed OMS–658 on the first occasion) on 29th June.

Spraymen were dressed in overalls, sou'westers and rubber boots and were all wearing masks while spraying or weighing the compounds. Before handling the insecticide the foremen and spraymen were given a lecture on the spraying technique and safety precautions, and they attended a practical training course.[1] Before and during each spraying cycle the spraymen were warned that they should notify us immediately if they experienced any discomfort. Washing facilities were provided and spraymen washed their hands frequently.

Toxicological studies

Clinical examinations of spraymen and exposed villagers were performed throughout the whole period.

In order to trace any adverse effects and evaluate the significance of cholinesterase inhibition plasma cholinesterase activity was determined in inhabitants before and after the spraying (Table 1) and in spraymen on 18 separate occasions throughout the entire period of spraying. In this study electrometric method as described by *Stubbs* and *Fales* (3) was employed (see later). The changes in cholinesterase activity are expressed as percentage of initial values, which were obtained before exposure to insecticides.

2-*iso*propoxyphenol was estimated in the urine of spraymen and inhabitants exposed to OMS–33 according to the method described by *Dawson et al.* (4). Urine samples were usually collected between 8 and 10 a. m. and their volume varied from 20 to 300 ml.

Methods of determining cholinesterase activity

For all assays blood samples were collected by finger prick. Special care was taken to remove traces of any possible insecticide from the skin by washing hands with soap and water and rubbing the finger tip thoroughly with a piece of gauze soaked in ethanol.

[1] Mr H. G. Smith, WHO Sanitarian, was in charge of the training and was responsible for correct application of insecticides.

Table 1

Dates of spraying and dates of blood collections in villages

Insecticide (and village) sprayed	Pre-exposure collection on	Insecticide sprayed on[1]	1st post-exposure collection on	2nd post-exposure collection on
OMS-658 *O,O*-dimethyl *O*-(4-bromo-2,5-dichlorophenyl) phosphorothioate (Oko-Eko)	21 and 22 May	25 and 26 May	1 June	29 June
OMS-43 *O,O*-dimethyl *O*-(4-nitro-*m*-tolyl) phosphorothioate (Ewu-Elepe)	10 June	11 June	17 June	—
OMS-33 2-*iso*propoxyphenyl *N*-methyl carbamate (Egbin)	16 June	22, 23 and 29 June	30 June	—

[1] Additional sprayings were performed with OMS-43 in Baiyeko village on 12th June, and with OMS-33 in Akessan village on 24th and 25th June. These resulted in prolonged exposure to the spraymen. No blood collections in villagers were performed in connection with these additional sprayings.

Electrometric method. Blood samples were collected into heparinized glass capillary tubes, and plasma cholinesterase activity was determined at 25° C as described by *Stubbs* & *Fales* (3). As a rule, samples were analysed the same day as collected, and only exceptionally the following morning. Except for centrifuging, the samples were kept cold, either in ice during transport or in the refrigerator at 4° C. The electrometric method was employed for toxicological studies and was taken as a referential method when different methods were compared.

Acholest method was performed as described by *Sailer* & *Brausteiner* (5). The assay was carried out in the air-conditioned laboratory (22–25° C) the same or the following day after blood collection. For all estimations the remainder of plasma left in the glass capillaries after electrometric cholinesterase assay was used. The results are expressed in cholinesterase-activity units according to the formula suggested by *Richterich* (6) which includes the correction for environmental temperature.

Since the quantity of the paper impregnated is proportional to the volume of plasma, and the ratio of paper to plasma remains the same if the test paper is pressed well between the two slides (7), homogenous drops of about 0.025 ml plasma – instead of 0.050 ml as prescribed by *Richterich* (6) – were deposited. By this volume about 2/3 of the test paper was engaged in the reaction.

Tintometric method was used as described by *Edson* (8) and modified by *Watson* & *Edson* (9). The modified technique enables the user to regulate the time of reading according to the environmental temperature and so obviates the need for »normal blood« to be run for each operation. Using the »Standard Lovibond Disc 5/30« which covers the range from 0–100% activity in steps of 12.5%, many readings necessarily fell in between two values indicated on the comparator. In such instances, instead of making the reading of »the nearest match«, further subdivision of the 12.5% steps was carried out by making the readings such as »slightly above 87.5%«, or »slightly under 100%« which were recorded as 87.5(+) or 100(—), respectively. In this way two more values could be arbitrarily evaluated between every two 12.5% steps. When calculating results, 12.5/3 was added to the readings marked (+) and 12.5/3 was substracted from those marked (—). Fresh indicator solution was prepared weekly, and fresh substrate solution prior to each determination.

The cholinesterase activity in villagers was determined on the spot under field conditions. Environmental temperature varied from 28–33° C. As soon as the maximum of 18 blood samples had been collected, the analysis of enzyme activity was performed, and only after determination had been completed a further collection of blood samples was continued.

Radiometric method described by *Wintheringham* & *Disney* (10) was modified twice by the same authors in order to simplify the field procedure (11, 12). In both modifications the commercially available (1-^{14}C-acetyl) acetylcholine at higher specific activity was used. As the second modification (12) using only half the substrate concentration appeared in the course of our work, the first modification (11) was abandoned. Experimental conditions for the original version and for both modifications are presented in Table. 2. All the equipment necessary for the assay was assembled in England by the authors of the method.

The radioactive substrate solution was kept at —20° C, except when fractions were taken out for the assay. During the six-week period 16 sets of experiments were performed using the same substrate solution. During this time a steady fall in the radioactivity of reference slides – from 695 to 475 c. p. m. – was observed.

Blood samples were collected in the field and kept in ice during transport. The analysis of samples was performed in air-conditioned laboratory within 4–6 hours.

Table 2

Experimental conditions of the three versions of radiometric method

Version	Concentration of acetylcholine (M)	Time of reaction (sec.)	Blood diluted in
Original (10)	1×10^{-4}	180	Michel's enzyme buffer (1 : 50)
1st modification (11)	1×10^{-4}	12	Michel's enzyme buffer (1 : 11)
2nd modification (12)	5×10^{-5}	20	1% KCl (1 : 11)

RESULTS

Effects of insecticides used

OMS–658. – Clinically no adverse effect was observed either in the spraymen or in the villagers exposed to the insecticide. Two days after the completion of spraying five out of eight spraymen made some complaints. These could hardly be associated with exposure to the insecticide and were most likely due to their remembering last year's experience with OMS–15 (1). It is important to note that there were no complaints during the two-day-spraying nor the first day after spraying.

Very slight depression of plasma cholinesterase was found in spraymen the day after spraying. The enzyme activity, which averaged 94.9%, returned to normal values within three days (Table 3).

Table 3

Plasma cholinesterase activities in eight spraymen exposed to OMS-658

Date	Exposure	Activity[1] (%)
23 May	weighing OMS-658 (3 hrs)	
25 May	spraying OMS-658 (4 hrs)	
26 May	spraying OMS-658 (2–5 hrs)	
27 May		94.9 (90.0–99.8)
30 May		101.7 (99.5–103.9)

[1] Mean with 95% confidence limits.

Slight depresion with statistically significant mean change of —8.0% plasma cholinesterase was found in exposed villagers one week after spraying. Five weeks after spraying this depression was less pronounced (—5.6%) and was no longer statistically significant (Table 4).

OMS–43. – No adverse effect was clinically observed either in villagers or in spraymen.

Table 4

Changes in plasma cholinesterase activities in villagers exposed to OMS-658 (calculated as percentages of the initial values)

Interval[1]	1 to 2	1 to 3	2 to 3
No. of obscrvations . .	77	39	40
Falls	55	24	14
Rises	19	11	26
No change	3	4	0
Mean change . . .	—8.0	—5.6	+4.8
95% confidence limits .	—5.7 to —10.3	+0.3 to —11.5	+11.0 to —1.4

[1] 1 = before spraying; 2 = one week after spraying; 3 = five weeks after spraying.

Table 5

Plasma cholinesterase activities in three spraymen exposed to OMS-43

Date	Exposure	Activity[1] (%)
10 June	weighing OMS-43 (5 hrs)	
11 June	spraying OMS-43 (3 hrs)	
	1–2 hrs after spraying:	87.7 (77.0–98.4)
12 June	spraying OMS-43 (5 hrs)	
	1–2 hrs after spraying:	93.7 (90.5–96.9
13 June		89.9 (86.9–92.9)
18 June		100.5 (99.0–102.0)

[1] Mean with 95% confidence limits.

Determination of plasma cholinesterase activity in three exposed spraymen (Table 5) showed depression of enzyme activity similar to that observed in 1963 when examinations were carried out on a larger group of spraymen (1). A slight inhibition was observed after the first day's spraying; the inhibition was even less pronounced after the second day's exposure.

As shown in Table 6, no apparent inhibition of plasma cholinesterase could be found in the villagers exposed to OMS–43 six days after spraying.

Table 6

Changes in plasma cholinesterase activities in villagers exposed to OMS-43 (calculated as percentages of the initial values)

Interval	6 days after spraying with OMS-43
No. of observations . . .	18
Falls	6
Rises	12
No change	0
Mean change	+4.0
95% confidence limits . .	+8.1 to —0.1

Table 7

Plasma cholinesterase activities and excretion of phenol derivatives in the urine in three spraymen exposed to OMS-33

Date	Exposure to insecticide	Cholinesterase activity (%)			Phenol derivatives (μl/ml)		
		Spraymen			Spraymen		
		1.	2.	3.	1.	2.	3.
	pre-exposure values:						
18 June	weighing OMS-33 (3 hrs)	100.0	100.0	100.0	48.1	10.6	10.0
19 June	weighing OMS-33 (5 hrs)						
20 June		98.8	95.1	101.4	33.1	10.0	75.0
22 June	spraying OMS-33 (5 hrs)	87.7[1]	86.4	102.9	83.7[1]	19.4	34.4
	1–2 hrs after spraying:						
23 June	spraying OMS-33 (5 hrs)	79.8	100.0	97.2	—	—	—
	1–2 hrs after spraying:						
24 June		92.1	84.0	92.8	58.1	37.6	53.1
25 June	spraying OMS-33 (4 hrs)	85.3	84.0	98.6	—	—	—
	1–2 hrs after spraying:						
26 June	spraying OMS-33 (4 hrs)	93.3	80.3	105.8	76.5	30.0	22.5
	1–2 hrs after spraying:						
1 July		103.5	100.0	105.0	20.0	20.6	47.5

[1] Sprayed most of the roofs that day.

OMS–33. – Clinical observations did not reveal any adverse effect either in spraymen or in exposed villagers.

Changes in plasma cholinesterase activity in spraymen exposed to OMS–33 are shown in Table 7. As the crew consisted of only three spraymen, and the effect of the insecticide on cholinesterase activity differed markedly from one sprayman to another, individual cholinesterase levels are shown instead of average values. The same table contains the results of phenol derivatives determinations which were performed on the urine of the spraymen during exposure to OMS–33.

Negligible inhibition of cholinesterase was found in the spraymen weighing the insecticide (three and five hours respectively for two days). In one of the spraymen a marked increase in excretion of phenol derivatives was found.

During the 5-hour spraying on the first day the weather was very hot (about 35° C underneath the corrugated roofs of the huts). Cholinesterase activity was determined 1–2 hours after spraying and only a slight depression could be found in two spraymen. A marked increase in excretion of phenol derivatives was found in the spraymen who had sprayed most of the corrugated roofs that day. During the following three days of spraying cholinesterase activity varied from 79.9 to 105%; neither a further decrease in enzyme activity nor a further increase in excretion of phenol derivatives could be established with certainty. Four days after the last exposure to OMS–33 cholinesterase activity was found to be within normal values in all the three spraymen (Table 7).

Plasma cholinesterase activity was determined in villagers before, and one or six days after spraying. As shown in Table 8, post-exposure values did not differ significantly from the initial pre-exposure values. Excretion of phenol derivatives determined before and six days after spraying of OMS–33 in 25 and 21 villagers respectively showed significantly different averages. Calculated with 95% confidence limits they were 17.3 (12.8–21.8) before, and 28.6 (22.5–34.7) μg/ml after spraying.

Table 8

Changes in plasma cholinesterase activities in villagers exposed to OMS-33 (calculated as percentages of the initial values)

Interval	1 day after spraying	6 days after spraying
No. of observations	10	16
Falls	7	6
Rises	2	10
No change	1	0
Mean change	—5.8	0.0
95% confidence limit	+0.1 to —11.7	—3.9 to +3.9

Comparison of methods for cholinesterase activity determination

Acholest method. – In the first experiment cholinesterase activity was determined in 47 unexposed villagers. The results obtained by Acholest method and those obtained by the electrometric method are compared in Table 9. A relatively high coefficient of variability was found with the Acholest method (28%); a satisfactory correlation (r = 0.76) was obtained between Acholest and electrometric method.

Table 9

Comparison of the Acholest and electrometric methods in determining cholinesterase activity in unexposed villagers

Method	N	$\bar{x}$	s	$S_{\bar{x}}$	Coefficient of variability (%)	Total range of results
Acholest	47	40[1]	11	1.6	28	22–69
Electrometric .	47	0.71[2]	0.13	0.02	18	0.49–1.01

Correlation between the methods: r = 0.76 (on 47 pairs of observations)

[1] Analysis performed at 25° C; activity units calculated according to the formula suggested by *Richterich* (6).
[2] Δ pH/hr.

In the second experiment Acholest method was tested in villagers exposed to OMS–33. The results obtained before and 1–6 days after the spraying of the village are shown in Table 10. Neither method revealed any statistically significant change in plasma cholinesterase activity. On this occasion a high correlation between the two methods was found, *r* being 0.91 and 0.80 for pre-exposure and post-exposure determinations, respectively.

Table 10

Comparison of the Acholest and electrometric methods in determining cholinesterase activity in villagers exposed to OMS–33

Method	Before spraying				1–6 days after spraying			
	N	$\bar{x}$	s	S_x	N	$\bar{x}$	s	$S_{\bar{x}}$
Acholest	35	32[1]	7.6	1.3	26	33[1]	8.2	1.6
Electrometric	35	0.73[2]	0.12	0.02	26	0.72[2]	0.13	0.03
Correlation between the methods	r = 0.91				r = 0.80			

[1] Analysis performed at 22° C; activity units calculated according to the formula suggested by *Richterich* (6).
[2] Δ pH/hr.

Tintometric method. Cholinesterase activity was determined on three occasions in the total of 66 unexposed villagers. The average activity and its standard error was 83.9 ± 0.5 (%). The coefficient of variability of the method was 9.9%.

The results of cholinesterase determination in villagers exposed to OMS–43 and OMS–33 are shown in Table 11. According to this method neither insecticide produced any significant change in cholinesterase activity.

Table 11

Tintometric method. Cholinesterase activity in villagers before and after exposure to OMS-43 and OMS-33

Insecticide	Pre-exposure determination			Post-exposure determination[1]		
	N	$\bar{x}$[2]	$S_{\bar{x}}$	N	$\bar{x}$[2]	$S_{\bar{x}}$
OMS-43	18	84.3	1.2	18	87.3	1.5
OMS-33	36	86.1	0.9	26	84.5	1.4

[1] Six days after the houses were sprayed with OMS-43; 1–6 days after the houses were sprayed with OMS-33.
[2] % activity according to Lovibond comparator.

Radiometric method. – In spite of the simplicity of the technical procedure difficulties were encountered when the analyses were performed on a larger scale for a longer period of time. These were mainly associated with (1) the use of complex electronic equipment and (2) the relatively small capacity of the batteries supplying the portable counting equipment. Several breakdowns of the scaler could not be repaired in time – in spite of spare parts being available – so that some determina-

Table 12

Radiometric method. Cholinesterase activity in unexposed villagers and in villagers exposed to OMS-33

Method	N	$\bar{x}$[4]	s	$S_{\bar{x}}$	Coefficient of variability (%)	Total range of results
1st Modification[1]	85	194	117	12.7	60	5–596
2nd Modification[2]	36	82	28	4.7	35	8–148
2nd Modification[3]	27	44	31	5.9	70	1–115

[1] Oko-Eko villagers, pre-exposure determination.
[2] Egbin villagers, pre-exposure determination.
[3] Egbin villagers, post-exposure determination.
[4] μM of hydrolyzed acetylcholine/hr/ml of blood.

tions of the samples collected had to be omitted. The working plateau of the voltage, necessary for obtaining the constant background counts, was comparatively short, so that replacement of the battery was unavoidable when a larger number of samples had to be counted. Since the voltage of freshly charged battery was always too high, it took 1–2 hours of counting the background repeatedly before the optimal voltage was reached. Only then could the battery be used reliably, but for not longer than about two hours. Recharging of the battery took eight hours.

Cholinesterase activity was determined by the first and second modification in 85 and 36 unexposed villagers respectively. The results of these analyses are shown in Table 12.

Large coefficients of variability were found especially for the first modification. Extremely wide total range of normal cholinesterase activity was obtained by either method. The results expressed in μM hydrolyzed acetylcholine/hr/ml of blood, ranged from 5 to 596 for the first and from 8 to 148 for the second modification.

In spite of the fact that the standard deviation of the pre-exposure values obtained by the second modification (28 μM), was too high a fraction of the average (82 μM) to apply statistics reliably, determination of post-exposure cholinesterase activity was undertaken. Examinations were carried out on 27 villagers 1–6 days after their houses had been sprayed with OMS–33. As shown in the same table the average cholinesterase activity and its standard error was 44 ± 5.9 μM of acetylcholine hydrolyzed, and the coefficient of variability was as high as 70%.

DISCUSSION AND CONCLUSIONS

OMS–658 and OMS–43 belong to the group of dimethyl phosphate esters. It is well known that prolonged exposure to organophosphorus compounds may produce a cumulative inhibitory effect on cholinesterase whenever the absorption of the compound is higher than the rate of reactivation of the inhibited enzyme and the rate of synthesis of the fresh enzyme. Although dimethylphosphorylated enzyme reactivates relatively rapidly (13) it has been shown that prolonged contact between the inhibitor and the cholinesterase *in vivo* results in the formation of the unreactivatable enzyme (14). When such a stage in the course of exposure is reached the synthesis of the fresh enzyme becomes the only recovery process which can compensate further absorption of the insecticide.

The fact that clinically no adverse effect was observed in spraymen who sprayed OMS–658 and OMS–43, and that only slight depression of cholinesterase activity was found during their 2-day-spraying operation, does not mean that the same negligible effects would be observed if the spraymen were exposed for several weeks. However, observations of

villagers exposed to OMS–43 in 1963 (1) and those exposed to OMS–43 or OMS–658, provide reliable indications that either insecticide – if sprayed under the same conditions – should prove sufficiently safe to exposed r e s i d e n t s. Consequently both insecticides can be introduced into extended field trial, which should provide additional data under conditions of prolonged spraying which are necessary to evaluate the risk to spraymen.

OMS–33 belongs to the carbamate group and was tested under more unfavourable conditions. Four-day-spraying was performed by three spraymen, 4–5 hours daily. On the first day weather conditions were similar to those under which 3-*iso*propylphenyl *N*-methylcarbamate was sprayed in 1963 (1). None of the spraymen, including an additional crew of eight men who were exposed for one hour, nor any villagers produced any complaints, symptoms or dermal reaction whatsoever. These observations indicate that OMS–33 differs markedly from 3-*iso*propylphenyl *N*-methylcarbamate which had to be withdrawn from the trial in 1963, because of its adverse effects on both spraymen and exposed residents (1).

As carbamylated cholinesterase reactivates very rapidly (15) and no formation of unreactivatable enzyme has been produced experimentally, a cumulative effect on cholinesterase is not expected. It can be anticipated, therefore, that the negligible effect of OMS–33 observed in spraymen during the four-day exposure would not become worse if a similar exposure were prolonged for several weeks. It is important that the total spraying hours per day should be limited, as it is much safer to spray a carbamate for a short daily period (4–5 hours of actual spraying) and to prolong the whole spraying programme.

Thus OMS–33 may be introduced into an extended field trial and gradually even into general use, bearing in mind that no satisfactory field method for measuring the degree of cholinesterase inhibition by carbamates has yet been developed (see later).

Similarly as in the trial with Carbamyl and 3-*iso*propylphenyl *N*-methyl carbamate (1) an increase in phenol derivatives excretion in urine was observed in spraymen and villagers exposed to OMS–33. Difficulties in collecting numerous urine samples in exact time relations exposure have been already pointed out (1).

Owing to the fact that in this trial only slight inhibition of cholinesterase activity was observed in spraymen and exposed villagers, comparison of different methods was limited to normal or slightly inhibited samples only, so that less sensitive methods could not be fully evaluated. At the same time it should be pointed out that a strict comparison was not possible, since some of the methods differed regarding the enzyme measured. By the electrometric and Acholest methods plasma cholinesterase activity was measured. In the case of the tintometric and radiometric methods whole blood activity was assayed; plasma cholinesterase activity was measured predominantly by the former and the erythrocyte

cholinesterase by the latter. Nevertheless, it was possible to evaluate the reliability and practicability of each method when used under field conditions.

The electrometric method performed under the well-equipped laboratory conditions served as a reference method.

The improved tintometric method gave reproducible results and its use in evaluation of exposure to organophosphorus insecticides under field conditions was fully justified.

In measuring exposure to the reversible inhibitors the Acholest method has the advantage of using undiluted plasma so that the degree of cholinesterase inhibition is not diminished by dilution (16). In this method retesting of the correction factor for the environmental temperature may be necessary, and a calibration which would allow interpretation of the results in terms of percentages of cholinesterase activity is desirable.

The radiometric method requires further development as too many erratic results were obtained when the analyses were performed on a larger scale for a longer period.

None of these methods seemed to be suitable for determining exposure to carbamate insecticides, and more work has to be done to develop a suitable field method for this group of compounds. The practical experience gained during a similar field trial (1) as well as recent studies on experimental carbamate poisoning (17) suggest that occupational over-exposure to carbamates will produce early warning symptoms long before a lethal dose had been absorbed. These »early warning« symptoms of carbamate intoxication might partly compensate for the lack of an »early warning« test of exposure which is readily available for the organophosphorus compounds in the form of existing field methods for determining blood cholinesterase activity.

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References

1. *Vandekar, M.:* Bull. Wld Hlth Org., *33* (1965) 107.
2. *Taylor, A.:* Bull. Wld Hlth Org., *29* (1963) 213.
3. *Stubbs, J. L., Fales, J. T.:* Amer. J. med. Technol., *26* (1960) 25.
4. *Dawson, J. A., Heath, D. F., Rose, J. A., Thain, E. M., Ward, J. B.:* Bull. Wld Hlth Org., *30* (1964) 127.

5. *Sailer, S., Braunsteiner, H.:* Klin. Wschr., *37* (1962) 986.
6. *Richterich, R.:* Schweiz. med. Wschr., *92* (1962) 263.
7. *Holmstedt, B.:* Communication to WHO, 1964.
8. *Edson, F. F.:* World Crops, *10* (1958) 49.
9. *Watson, W. A., Edson, F. F.:* Med. Dept. Chesterford Park Research Station, Mimeographed report, TOX. 115.
10. *Winteringham, F. P. W., Disney, R. W.:* Bull. Wld Hlth Org., *30* (1964) 119.
11. *Winteringham, F. P. W., Disney, R. W.:* Unpublished.
12. *Winteringham, F. P. W., Disney, R. W.:* Lab. Practice, *13* (1964) 739.
13. *Aldridge, W. N.:* Biochem. J., *54* (1953) 442.
14. *Vandekar, M., Heath, D. F.:* Biochem. J., *67* (1957) 202.
15. *Wilson, I. B., Harrison, M. A., Ginsburg, S.:* J. biol. Chem., *236* (1961) 1498.
16. *Churchill-Davidson, H. C., Griffiths, W. J.:* Brit. med. J., *2* (1961) 994.
17. *Vandekar, M., Reiner, E., Svetličić, B., Fajdetić, T.:* Brit. J. industr. Med., *22* (1965) 317.

Sadržaj

ISTRAŽIVANJE TOKSIČNOSTI TRIJU ANTIKOLINESTERAZNIH INSEKTICIDA PRILIKOM NJIHOVA TERENSKOG TESTIRANJA I UPOREĐENJE METODA KORIŠTENIH ZA ODREĐIVANJE AKTIVNOSTI KOLINESTERAZA

U okviru programa Svjetske zdravstvene organizacije izvršena su u toku 1964. godine pri Jedinici za testiranje insekticida u Lagosu, Nigerija, terenska toksikološka istraživanja dvaju organofosfornih i jednog karbamatnog insekticida: *0,0*-dimetil *0*-(4-bromo-2,5-diklorofenil) fosforotioat (OMS-658); *0,0*-dimetil *0*-(4-nitro-*m*-tolil) fosforotioat (OMS-43); 2-*izo*propoksifenil *N*-metil karbamat (OMS-33). U toku prskanja insekticida promatran je njihov učinak na radnike-prskače kao i na stanovnike tretiranih nastambi. Nijedan od upotrijebljenih spojeva nije izazvao klinički zamjetljivih toksičnih pojava, a samo je jedan insekticid doveo do neznatnog pada aktivnosti kolinesteraze plazme eksponiranih ljudi.

Aktivnost kolinesteraze prskača i eksponiranih domorodaca određivana je elektrometrijski, a na domorocima izvršeno je i komparativno testiranje još triju metoda: Acholest-metode, tintometrijske i radiometrijske metode. Kritički su iznesene prednosti i nedostaci pojedine metode i dana je ocjena njihove vrijednosti kad se koriste u terenskim uvjetima.

Ni jedna od postojećih metoda ne može se, zbog karakteristika enzima inhibiranog karbamatima, pouzdano primijeniti za ocjenu ekspozicije toj grupi insekticida. Autori smatraju da se manje otrovni karbamatni insekticidi, kao što se pokazao OMS-33, mogu ipak upotrijebiti za opsežnija terenska istraživanja pri sličnim uvjetima ekspozicije. Na osnovu terenskih iskustava i rezultata eksperimentalnih istraživanja može se s pravom očekivati da će karbamatni insekticidi – za razliku od organofosfornih spojeva – izazvati kliničke znakove prekomjerne apsorpcije mnogo prije nego što je u organizam doprla po život opasna količina otrova. Pri ekspoziciji karbamatima, spomenuti »rani simptomi upozorenja« mogu u određenoj mjeri kompenzirati nedostatak »ranog testa prekomjerne apsorpcije«, kakvi postoje za ocjenu ekspozicije organofosfornim insekticidima.

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